A SLIME BACTERIUM FROM THE PEACH, ALMOND AND CEDAR.

(*Bacterium persicæ*, n.sp.).

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During the examination of specimens of peaches affected with gum-flux, there was isolated a bacterium which produced a slime upon the surface of solid media containing saccharose. When recently separated and infected upon saccharose-potato agar, it produced a growth which, upon the 4th day at 22° C., was like a heap of diminutive white sausages, the individuals being clearly seen imbedded in a transparent jelly. After the fourth day the growth became convoluted, then flat as the slime became less viscous; the slime then slowly gravitated down the sloped agar surface. Growth was most rapid at 37° C., at which temperature the culture had the character of stiff flour-paste. The phenomenal appearance was interesting, but unfortunately for purposes of diagnosis, later cultures failed to produce the curious growth and simply developed as an uncharacteristic white, raised expansion.

A quantity of the slime was prepared by growing the organism upon plates of saccharose-potato agar, from the surface of which it was readily removed. It had a loose, pasty consistency and formed a white emulsion with water. Upon the addition of alcohol the slime was coagulated and could be strained through calico and squeezed. After the removal of the saccharose and reducing sugars, an attempt was made to separate the constituents

of the slime by heating the emulsion under pressure in the autoclave, a method which had been very successful in the separation of the constituents of the arabin bacterial slimes. The attempt failed, and no separation of the gum could be induced by the method.

In view of the probable impossibility of obtaining a clear solution of the gummy constituent, the whole slime was repeatedly coagulated with alcohol until most of the salts had been removed and the bulk of the slime remained suspended in the dilute alcohol as an opalescent solution. Saline flocculating agents were then added. First potassium chloride threw down a fraction, then strong alcohol precipitated a second fraction, barium chloride flocculated a third portion. The mother liquor was now clear and bright, but on boiling off the alcohol a fourth fraction settled out. All these fractions, with the exception of the last, formed emulsions with water; the last fraction was more of the nature of a suspension. The emulsions and the suspension behaved to reagents in a manner precisely similar to the original slime and to the residue which was not "milked" by the dilute alcohol. From the similar behaviour of the fractions it was evident that the slime contained but one gum constituent. Coagulation of the emulsions was effected by alcohol, neutral and basic lead acetates, milk of lime and baryta water. These reactions were constant with the slime from the several races of the bacterium. Coagulation was also effected by other reagents, but the reactions could not be depended upon even with slimes from the same race. For example, slime grown at 37° gave a precipitate with 1 % and 10 % copper sulphate, while when grown at 18° no precipitate was obtained. With the crude slime a precipitate was obtained with ferric chloride, but the partly purified slime gave no precipitate. The ready solubility of the slime carbohydrate in dilute acids may account for the irregular behaviour with the salts of the metals.

Of more importance than the reactions of the slime is the nature of the essential carbohydrate. From saccharose, bacteria can produce dextran, levan, galactan, arabinan-galactan and deriva-

tives of other sugars. The nature of the gum is ascertained from the sugar which it produces upon hydrolysis. The sugar is most readily determined by means of the osazones in cases of bacterial gums and slimes, when other bacterial by-products are present and when the quantity of material is usually small. The solubility, the appearance, and the melting points of the osazones are usually very characteristic.

The slime was repeatedly dissolved in water and precipitated with alcohol until a portion when hydrolysed at 70° C. with dilute hydrochloric acid showed the absence of reducing sugars. The hydrolysis of the gummy constituent was effected by boiling the slime with 5 % sulphuric acid for six hours. A slight humus-like deposit was filtered off and the sulphuric acid was removed by treatment with barium carbonate. From the clear filtrate the barium salt of an inorganic acid was removed by alcohol. The alcohol was distilled off and the solution after clarification with aluminium hydrate was evaporated to small volume. The preparation and separation of the osazones will be more readily followed from the table on the next page.

From the results set down in the table it is seen that the sugars into which the essential carbohydrate of the slime hydrolyses are arabinose and galactose. In these sugars the galactose greatly predominated, the arabinosazone having been obtained in relatively small quantity.

In slimes which are obtained by growing bacteria upon the surface of agar, there is always a danger of portions of the medium getting into the slime. When large covered dishes are used, drops of condensed water gather upon the cover and, unless removed by sloping the cover as they form, may fall into the solidifying medium, which is softened at that place and readily comes away with the slime. The traces of agar, however, which are thus accidentally gathered do not appear to influence the determination of the constituents of the slime, probably because the agar, which consists chiefly of pararabin, is not hydrolysed by boiling with 5 % sulphuric acid—at least in the time (6-10 hours)

Preparation and Separation of the Osazones.

Slime boiled with 5 % H_2SO_4, neutralised with $BaCO_3$, filtered, clarified with $Al(OH)_3$, evaporated to small volume. Added 2 c.c. phenylhydrazine solution, heated on waterbath for 15 minutes, filtered through hot, wet filter, rejected tarry residue, cooled filtrate. Filtered osazone and washed with cold water.

Mother liquor heated for an hour after further addition of 2 c.c. phenylhydrazine solution, cooled, filtered. Filtrate rejected. Osazone washed into beaker, diluted to 20 c.c., boiled, filtered through hot, wet filter, washed with warm water.

Osazone washed into small beaker with 10 c.c. water, added 1 drop phenylhydrazine solution, heated for 30 min., filtered hot.

Filtrate cooled, filtered. Osazone with characters of arabinosazone and m.p. 158-159°.

Osazone m.p. 175-178°.

Osazone dried on porcelain, extracted with ether, dried at 100°. m.p. = 185°.

Filtrate cooled, filtered; residue dried on porcelain, extracted with ether, dried at 100°; osazone m.p. 160-163°; quantity too small to separate arabinosazone.

These added together; the mixture was practically insoluble in boiling water; added alcohol slowly while on water bath until most of the osazone had dissolved, filtered.

Residual osazone dissolved in hot alcohol, cooled, filtered, washed with cold dilute alcohol.

Filtrate cooled when osazone separated out, filtered.

Osazone m.p. 193-194° with characters of galactosazone.

Boiled off alcohol from filtrate, filtered; residual osazone m.p. 193-194° with characters of galactosazone.

Osazone m.p. 191-192°.

Filtrate boiled to expel alcohol, residue obtained m.p. 171-173°; quantity too small to separate further.

usually occupied in hydrolysis. I have tested the hydrolysed products of a glucose-yielding slime grown upon agar and have failed to detect arabinose. The probability of the agar contributing to the products of the hydrolysis of this slime is therefore remote.

The slime can also be obtained, though in comparatively small quantity, by growing the bacterium in fluid media containing saccharose. A solution containing saccharose 50, peptone 2, ammonium chloride 1, potassium phosphate 1, magnesium sulphate 0·5, chalk 10, and water 1000 was prepared, and after sterilisation and infection with the organism, it was kept at the laboratory temperature (22-25°). In 10 days the medium had become ropy and had the consistency of white of egg. The opalescent, supernatant liquid *which strongly reduced Fehling's solution*, showing the presence of invertase, was decanted from the sediment, tested with a few drops of hydrochloric acid and finally coagulated with alcohol. A springy coagulum which rapidly collected round the stirring rod and a slow settling flocculent precipitate were formed. The coagulum was separated from the flocculent precipitate and both were repeatedly treated with water and with alcohol until the sugars had been eliminated. The characters of the alcoholic precipitates were maintained throughout these operations, and upon treating the precipitates with water a gummy solution and an insoluble swollen portion was always obtained. The soluble gum of both portions behaved similarly in being coagulated with or precipitated by the basic and neutral acetates of lead, baryta water and milk of lime, so that the gums were apparently identical. There was a difference in the viscosity of the solutions; that obtained from the coagulum was always more viscous than that obtained from the flocculent precipitate. In spite of this the amounts of the precipitates formed upon the addition of the reagents were greater in the solution from the flocculent precipitate than in the solution from the coagulum. The increased viscosity of the solution which appeared to contain more gum was probably due to the presence of a greater quantity of the albuminoid products of the bacteria.

By using aluminium hydrate a clarification of the gummy solutions was effected, and although this reagent also removed some of the gum, yet the clear solutions were still viscous. These solutions were neutral to litmus paper, and upon being tested were found to be inactive to polarised light.

The slime thus obtained in saccharose solutions, and therefore free from any admixture with agar, was hydrolysed with dilute sulphuric acid after all saccharose and reducing sugars had been eliminated. The crude osazone was extracted with ether and then dissolved in 85% alcohol to remove an unhydrolysed product. The osazone obtained upon evaporating the alcoholic solution to dryness melted at 181-182° and appeared microscopically to consist of two kinds of crystalline groups, one being pale yellow, the other dark yellow in colour. Hot water extracted a constituent which upon evaporation appeared as a brown deposit and which melted at 158-159°, the melting point of arabinosazone. Thus arabinose is proved to be a constituent of the hydrolysed carbohydrate and was not in the former tests derived from the agar upon which the slime was produced.

The gum is one of those soluble kinds which readily become altered to an insoluble modification upon drying or by the action of dehydrating agents such as alcohol. The insoluble modification is soluble in dilute acid and insoluble in dilute alkali. It is therefore akin, so far as the solubility is concerned, to the metarabin and pararabin gums. But unlike these gums, it is not readily converted from the insoluble to the soluble modification, and cannot therefore be of any direct commercial importance.

The bacterium undoubtedly contributes a part of the natural gum of the plants in the tissues of which it occurs, but the part is so small as to be almost negligeable. I obtained some almond gum from Mr. Stoward, of Adelaide, and removed the soluble arabin by soaking the gum in water and filtering. The insoluble metarabin was dissolved by heating under pressure, and after acidification with hydrochloric acid the gum acids were precipitated with alcohol. The acid alcoholic solution was then neutral-

ised with sodium hydrate, when a precipitate settled out. This was treated with water and filtered. The solution, which was neutral to litmus, was coagulated by alcohol and precipitated by barium hydrate (not by barium chloride), neutral lead acetate and basic lead acetate. These precipitates were curdy, like other gum precipitates, and when considered in conjunction with the method of separation (*i.e.*, the solubility of the carbohydrate in acid alcohol) show that the constituent had been produced by the bacterium.

Hitherto the slime had been formed on media or in solutions containing saccharose without which no pronounced formation of slime occurred. Other sugars and carbohydrates had not, however, been tested, and therefore experiments were made to determine what other substances could replace saccharose. To dilute potato-extract agar, simple peptone agar and ordinary nutrient agar, small quantities of the following substances were added: saccharose, levulose, dextrose, galactose, maltose, lactose, raffinose, mannite, starch, inulin, dextrin and glycerine. The potato-extract medium did not give results so sharply as the ordinary nutrient agar, probably because that medium contains reducing sugars and other substances that assist gum-formation. They, however, served to corroborate the results obtained with ordinary meat-extract-peptone agar and simple peptone agar. Slime was produced from all the substances except lactose, starch and inulin.

Carbon dioxide was imperceptibly evolved during the slow fermentation of saccharose. Its presence in the air of the culture flask was shown by drawing the air above a 5 days' culture through baryta water contained in an attached flask. The usual precautions were taken to exclude aerial carbon dioxide when the medium was infected, and it is needless to say that carbonates were absent from the medium.

The acids that are produced from saccharose simultaneously with the gum were found to consist chiefly of lactic and butyric, with traces of succinic, acetic and formic. The ratio of volatile

to non-volatile acids was as 1 : 4. The acids were detected by the scheme which has already been described.*

Ethyl alcohol is also a by-product in the fermentation. A few drops were obtained by repeatedly distilling the fluid of a chalk culture after it had been saponified with barium hydroxide. The alcohol gave the iodoform reaction, burned with a blue flame and boiled at 78° C.

The organism is a non-motile, spore-bearing bacterium, and beyond the formation of slime and the secretion of invertase it has no distinctive characters. It may be related to *Bac. mucosus*, Zimm., or to *Bact. glutinosum*, Kern, but as the formation of a similar gum or slime by these bacteria has not been described, and as this is the chief and important characteristic of the bacterium, it must be accepted as new until such time as it is proved that other bacteria with approximate cultural characters can produce a chemically identical gum. Since the organism was in the first instance obtained from the peach, I have named it *Bacterium*† *persicæ*.

Although obtained originally from the peach, it may occur in other fruits and plants. A race which when freshly isolated produced a spotted instead of the sausage appearance upon saccharose-potato agar was obtained from a specimen of red cedar, *Cedrela australis*, F.v.M., affected with gum-flux. Another race which produced a homogeneous white slime was found in almonds which were exuding gum. These races had slight differences when grown upon various media, and in the list of cultural characters which is appended these differences are indicated.

Bacterium persicæ, n.sp.

Shape, &c.—Thick large spongy rods with rounded ends generally grow in chains; occasionally a few clostridium forms are seen. The size of the individual rods are 1·2-1·5 : 3-6 μ; as observed in the hanging drop, they measure 1·5 : about 7·5 μ.

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* These Proceedings, 1903, i., 118-120.

† According to Migula's classification.

The rods are devoid of motility, and no flagella could be detected. The spongy rods are decolorised in places by the Gram method. The spores are central and oval, and measure 1 : 1·5 μ; germination is polar.

Temperature, &c.—The bacillus grows equally well at 30° and 37°, at 15° and 22° the growth is less. It is aërobic and does not grow under anaërobic conditions such as beneath a mica sheet in plate culture.

Nutrient agar plate.—The colonies in 24 hours at 30° are white, raised, dry and rounded. Microscopically they are clouded and hatched. The deep colonies are opaque, irregular and fibrous. Upon the second day the margin of the colony has become puckered, and microscopically the colonies appear granular, with a margin like a yeast colony.

Saccharose-potato agar plate.—At 22° the colonies are in 48 hours translucent white and raised. They become white, appearing like drops of flour paste, and when free to grow soon reach a centimetre in diameter. Microscopically the translucent white colonies are either clouded or opaque. The deep colonies are irregular and opaque.

Nutrient gelatine plate.—The colonies consist of a white felted or floccose mass in a crateriform liquefied area. The deep colonies are irregular, rough and opaque.

Nutrient agar stroke.—The growth is raised, white or buff-white, dry, glistening or fatty, lobular and rough, with microscopic puckerings. It becomes broad and translucent, the roughness disappears and the culture becomes flat and speckled. The edge is at first smooth, but becomes ciliate and the medium darkens.

Saccharose-potato agar stroke.—A very luxuriant white or dirty white slime is formed. It slowly gravitates, generally producing vertical furrows. At 22°, and when recently isolated, the growth may show a sausage-like, wisp-like or wrinkled structure which becomes homogeneous.

Nutrient gelatine stab.—The growth is faint and filiform below, with a tubular liquefied area above and an air bubble at the top.

The liquefied area becomes napiform and shows clear, opalescent and white portions.

Glucose-gelatine stab.—The medium is partly consumed and partly liquefied, showing an air bubble and a crateriform liquefied area bearing a film. The needle track below the liquefied area is white and filiform. The liquefied area becomes funicular and the sunken film becomes wrinkled. No gas is produced in the body of the medium.

Potato.—The growth appears as white or yellow-white, dry, dull or glistening crusts; these fuse together to form a wrinkled expansion which ultimately becomes pasty.

Bouillon.—The medium is clear or faintly turbid; a loose flocculent deposit and broken surface ring is formed. The indol reaction was obtained. In-nitrate-bouillon, the nitrate is not reduced.

Milk.—The medium is partly, then completely peptonised, the reaction being faintly alkaline. The milk is not made ropy.

Summary.—From the peach, the almond and the cedar, races of an organism, *Bacterium persicæ*, n sp., were separated. The organism produced a slime when grown upon solid media or in fluid media containing saccharose. When grown upon solid media the saccharose could be replaced by many other carbohydrates and by glycerine. The essential carbohydrate of the slime was soluble in water, but upon drying became readily altered to an insoluble modification. The carbohydrate hydrolysed to arabinose and galactose, the latter predominating. The carbohydrate occurred in small amount in the gum exuded from one of the trees in which the organism was found. Besides forming the galactan-arabinan gum, the organism inverted the saccharose and produced ethyl alcohol, carbon dioxide, lactic, butyric and traces of succinic, formic and acetic acids.

Although the carbohydrate hydrolyses to arabinose and galactose, I do not consider that it belongs to the arabin group. The gums of this group are, by treatment with water in the autoclave at three atmospheres' pressure, readily and completely dissolved. I have found this to be the case with metarabin and with par-

arabin (as I shall show in a subsequent paper), and of course it holds for the soluble arabin. I do not wish it to be inferred that this behaviour is peculiar to the arabin gums. It is not, for as I have shown, the gum of *Bact. sacchari* is dissolved by the treatment and this, as I shall show in a future paper, is a gelatine gum. But on account of the divergence from the recognised members of the group I have not included this paper in my series of papers upon "The Bacterial Origin of the Gums of the Arabin Group."